Table S1. Cell volumes of log phase (n=30) and stationary phase cells (n=5).

Phase	Cell No.	Cell Volume [a] (μm^3)	Cytoplasm Volume [b] (μm^3)	Periplasm Volume [c] (%)
Log phase	1	0.03297	0.01982	40
	2	0.04903	0.03880	21
	3	0.04097	0.02908	29
	4	0.05568	0.03483	37
	5	0.05761	0.04218	27
	6	0.03206	0.01992	38
	7	0.03822	0.02560	33
	8	0.04066	0.02931	28
	9	0.03132	0.01822	42
	10	0.02834	0.01616	43
	11	0.04545	0.03150	31
	12	0.03332	0.02191	34
	13	0.04222	0.02491	41
	14	0.04196	0.02387	43
	15	0.05773	0.03828	34
	16	0.03058	0.02002	35
	17	0.03003	0.02170	28
	18	0.04816	0.03036	37
	19	0.01463	0.00727	50
	20	0.03536	0.02086	41
	21	0.03817	0.02338	39
	22	0.02980	0.01991	33
	23	0.02863	0.01922	33
	24	0.03270	0.01675	49
	25	0.03541	0.02475	30
	26	0.03291	0.02058	37
	27	0.03043	0.01530	50
	28	0.02206	0.01284	42
	29	0.05447	0.03192	41
	30	0.02396	0.01560	35
	Mean ± SD	0.03716 ± 0.01063	0.02383 ± 0.00811	36.7 ± 7.0
Stationary phase	1	0.02867	0.01391	51
	2	0.02619	0.01376	47
	3	0.03665	0.01858	49
	4	0.03991	0.01077	73
	5	0.04182	0.01631	61
	Mean ± SD	0.03465 ± 0.00690	0.01467 ± 0.00294	56.2 ± 10.8

a. Cell volume is the total volume enclosed by outer membrane.

b. Cytoplasm volume is the volume enclosed by inner membrane.

c. Periplasm volume percentage is the ratio between periplasm volume (the volume between inner and outer membrane) and total cell volume.

Table S2 Statistic of periplasmic width in log phase cells. Width at cell body is measured at the narrowest part, and width at cell pole is measured from the widest part.

Cell No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean ± SD
Width at body (nm)	16	26	16	17	19	14	15	16	19	14	19	14	17	17	19	15	17	18	22	15	17.3 ± 2.9
Width at pole (nm)	55	35	165	41	32	25	26	38	25	27	31	31	67	26	38	28	28	40	65	127	47.5 ± 36.4

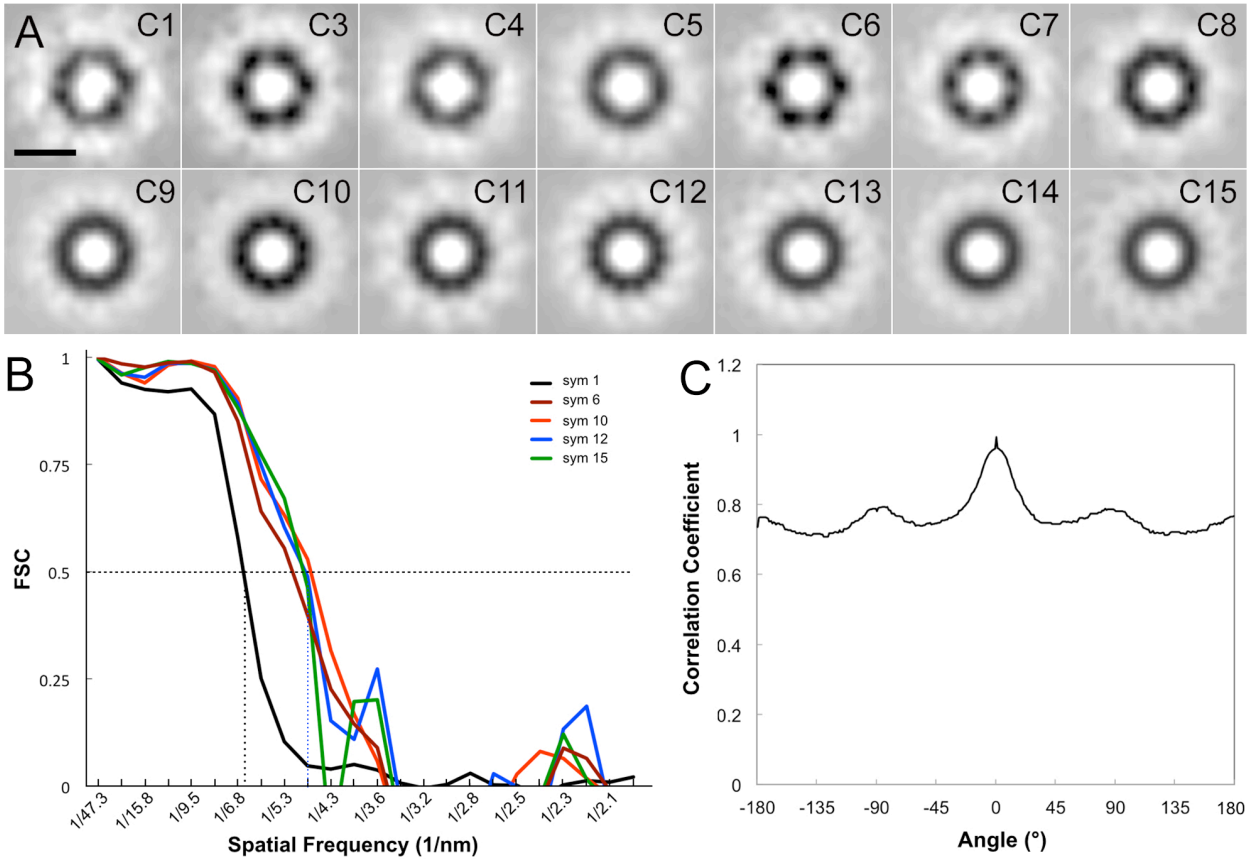

Fig. S1 Evaluation of possible rotational symmetries for the *Pelagibacter* PilQ pore complex. (A) Tomographic slices show the PilQ pore complex without symmetry (C1) and with applying 3- to 15-fold symmetry (C3-C15). (B) The extracted subtomograms of PilQ were divided into two independent datasets that were separately aligned against the same reference, before calculating the gold standard FSC curve. The resulting resolution is ~6.4 nm for non-symmetrized density map and ~4.7 nm for symmetrized density maps, as determined by the 0.5 Fourier shell correlation (FSC) criterion; note that the resolution is not sufficient to unambiguously determine the pore-symmetry of the native complex. (C) Real space correlation rotation analysis of non-symmetrized PilQ pore complex. The rotational correlation was done by calculating the correlation coefficient between the rotated map (rotate map from -180° to 180° with 1° increment) and the original averaged map. The peaks at four-fold symmetry for the

Pelagibacter PilQ pore complex would be consistent with the previously reported 12-fold quasi-symmetry of secretins. Scale bar = 10 nm in (A).

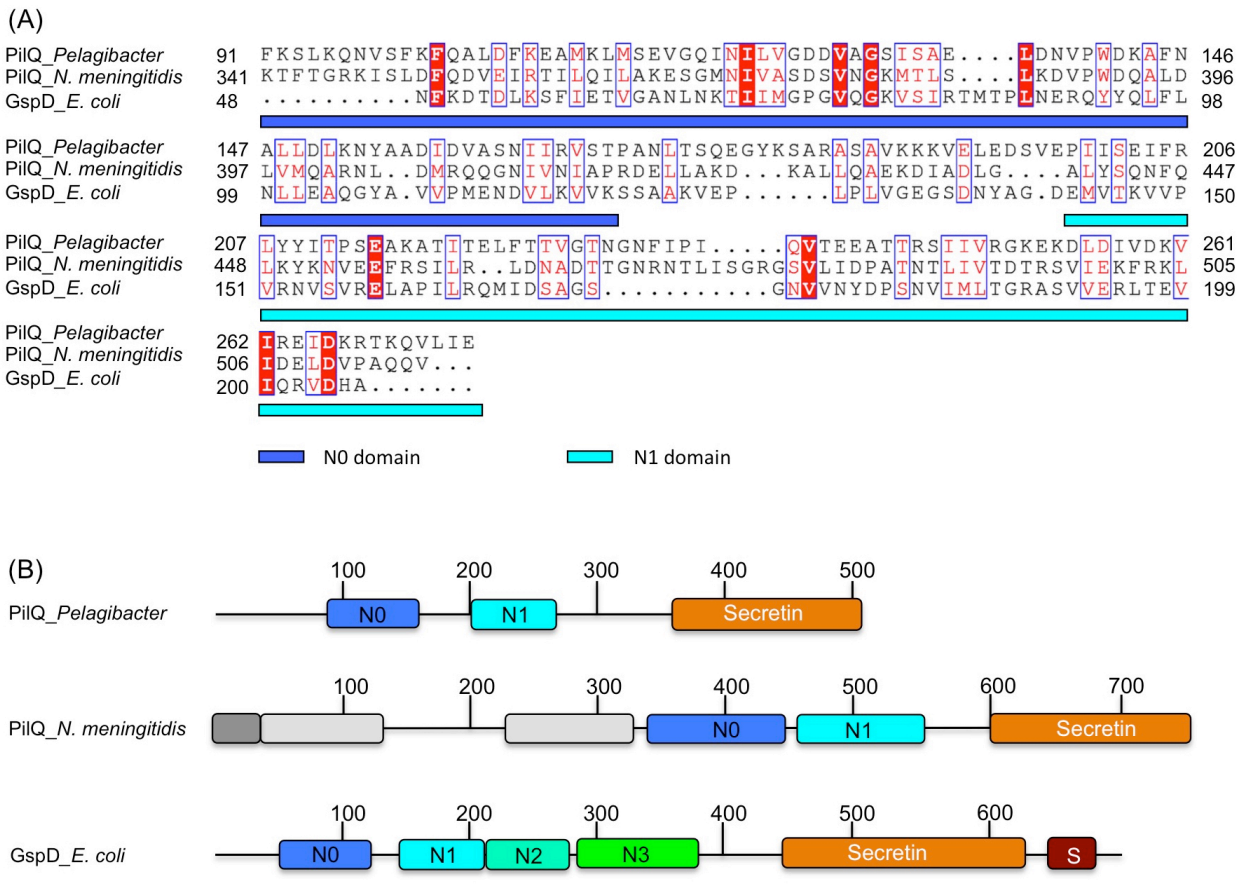

Fig. S2 Sequence alignment and domain organization of secretins in *Pelagibacter*, *Neisseria meningitidis* and *Escherichia coli*. (A) Sequence alignment of the N-terminal domains of *Pelagibacter* PilQ, *N. meningitidis* PilQ, and *E. coli* T2SS GspD was performed using Cluster Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). The alignment suggests that the N0 and N1 domains are also present in *Pelagibacter* PilQ. The assignment of N0 and N1 domain in *N. meningitidis* PilQ and *E. coli* GspD are based on their crystal structures, PDB: 4AV2 (PilQ) and 3EZJ (GspD). (B) Domain organization of the secretins in these three bacteria. In *Pelagibacter* PilQ secretin, 91-169 a.a. form N0 domain, 199 - 275 a.a. form N1 domain, and 368 – 518 a.a. form the C-terminal secretin domain.

Movie S1) Cell tomogram and 3D modeling of a dividing cell with type-IV pili. The first part of the movie shows sequential slices through the tomographic reconstruction of a *Pelagibacter* cell (also shown in Fig. 5E). Many type-IV pili project from (or close from) the division site into the extracellular space; some of the pili extended far away and were attached to the carbon support film on the EM grid. The second part of the movie shows the same cell as graphical 3D model (as shown in Fig. 5G) to provide an overview of the cell architecture of *Pelagibacter*.